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TITLE: Transgenic transchromosomal rodents for making human antibodies

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ABSTRACT:

The present invention provides novel transgenic nonhuman mammals capable of producing human sequence antibodies, as well as methods of producing and using these antibodies.

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Summary of Invention Paragraph - BSTX (6):
[0004] The invention provides a transgenic nonhuman mammal comprising two human immunoglobulin loci, wherein one of two said human immunoglobulin loci is a human heavy chain locus and the other locus is a human light chain locus; and wherein only one of said loci is of a transchromosome. In some transgenic nonhuman mammals, the transchromosome is **autonomous**. In some transgenic nonhuman mammals, the transchromosome comprises a fragment of human chromosome 14. In some transgenic nonhuman mammals, the human light chain locus is associated with an endogenous mammalian chromosome. In some transgenic nonhuman mammals, the human heavy chain locus is of a transchromosome and the human light chain locus is associated with an endogenous mammalian chromosome.

In some such transgenic nonhuman mammals, at least a part of the human light chain locus is cloned into a YAC vector. In some transgenic nonhuman mammals, the human heavy chain locus is comprised in hCF(SC20) and the human light chain locus is comprised in the human kappa light chain locus transgene KCo5. In some transgenic nonhuman mammals, the human light chain locus is of a transchromosome and the human heavy chain locus is associated with an endogenous mammalian chromosome. In some transgenic nonhuman mammals, the transgenic nonhuman mammal is a mouse. In transgenic nonhuman mammals, the endogenous mammalian heavy chain locus and at least one mammalian light chain locus are inactivated. In some such transgenic nonhuman mammals, the endogenous mammalian heavy chain locus and kappa light chain locus are inactivated.

Detail Description Paragraph - DETX (63):

[0095] The term "vector" is intended to refer to a nucleic acid molecule capable of transporting another nucleic acid to which it has been linked. One type of vector is a "plasmid", which refers to a circular double stranded DNA loop into which additional DNA segments may be ligated. Another type of vector is a viral vector, wherein additional DNA segments may be ligated into the viral genome. Certain vectors are capable of **autonomous** replication in a host cell into which they are introduced (e.g., bacterial vectors having a bacterial origin of replication and episomal mammalian vectors). Other vectors (e.g., non-episomal mammalian vectors) can be integrated into the genome of a host cell upon introduction into the host cell, and thereby are replicated along with the host genome. Moreover, certain vectors are capable of directing the expression of genes to which they are operatively linked. Such vectors are referred to herein as "recombinant expression vectors" (or simply, "expression vectors"). In general, expression vectors of utility in recombinant DNA techniques are often in the form of plasmids. In the present specification, "plasmid" and "vector" may be used interchangeably as the plasmid is the most commonly used form of vector. However, the invention is intended to include such other forms of expression vectors, such as viral vectors (e.g., replication defective retroviruses, adenoviruses and adeno-associated viruses), which serve equivalent functions.

Detail Description Paragraph - DETX (117):

[0149] Human antibodies against a variety of antigens can also be produced from non-human transgenic mammals comprising human immunoglobulin loci. Typically these immunoglobulin loci can encode substantially human sequence antibodies, preferably 95% or more identical to human sequences, more preferably 98-99% or more identical, and most preferably 100% identical. The immunoglobulin loci can be rearranged or unrearranged, and can comprise deletions or insertions relative to the natural human immunoglobulin loci. The loci can include genetic elements (e.g., non-coding elements such as enhancers, promoters, and switch sequences, or coding elements such as mu constant region gene segments) from other species, and from non-immunoglobulin loci, that do not contribute substantially to the coding portion of secondary repertoire (non IgM) antibodies. Preferred human immunoglobulin loci undergo DNA sequence alterations including V(D)J joining, heavy chain class switching, and somatic mutation in lymphoid cell and/or lymphoid cell precursors in the non-human

transgenic mammal to produce high affinity human antibodies to predetermined antigens. The human immunoglobulin loci contained in these transgenic mammals preferably include unrearranged sequences of natural human heavy and human light chain loci. Usually, the endogenous immunoglobulin locus of such transgenic mammals is functionally inactivated (U.S. Pat. No. 5,589,369; Takeda, S. et al., 1993, EMBO J. 12:2329-2366; Jakobovits, A., et al., 1993, Proc. Natl. Acad. Sci. U.S.A. 90:2551-2555; Kitamura, D. and Rajewsky, K., 1992, Nature 356: 154-156; Gu, H. et al., 1991, Cell 65:47-54; Chen, J. et al., EMBO J. 12:821-830; Sun, W. et al., 1994, J. Immunol 152:695-704; Chen, J. et al., 1993, Intl. Immunology 5:647-656; Zou, X. et al., 1995, Eur. J. Immunol 25:2154-2162; Chen, J. et al., 1993 Intl. Immunology 5:647-656; Boudinot, P., et al, 1995, Eur. J. Immunol. 25:2499-2505; Chen, J. et al., 1993, Proc. Natl. Acad. Sci. 90:4528-4532; Roes, J. and Rajewsky, K., 1991, Intl. Immunology 3:1367-1371; Gu, H. et al., 1993, Cell 73:1155-1164; Taki, S. et al., 1993, Science 262: 1268-71; Kitamura, D. et al., 1991, Nature 350:423-6; Lutz, C. et al., 1998, Nature 393:797-801; Zou, Y. et al, 1994, Current Biology 4: 1099-1103; Chen, J. et al., 1993, EMBO J. 12:4635-4645; Serwe, M. and Sablitzky, F., 1993, EMBO J. 12:2321-2327; Sanchez, P. et al., 1994, Intl. Immunology 6:711-719; Zou, Y. et al., 1993, EMBO J. 12:811-820). Inactivation of endogenous immunoglobulin genes preferably can be achieved, e.g., by targeted homologous recombination. The exogenous human immunoglobulin loci can be associated the endogenous mouse chromosomes or can be of (e.g., part of, inserted within or attached to) an introduced transchromosome.

Transchromosomes are introduced into a cell as a nonendogenous chromosome or chromosome fragment having a centromere and two telomeres. These transchromosomes commonly comprise telomere and centromere sequences and can comprise deletions relative to the parental intact chromosome.

Transchromosomes can also comprise additional inserted sequences. For example, two human immunoglobulin loci can be combined onto a single transchromosome by inserting sequences of a first immunoglobulin locus (e.g. from a YAC clone, a transchromosome, or an intact chromosome) into a transchromosome comprising a second immunoglobulin locus. This process can also be repeated to combine all three human immunoglobulin loci onto a single transchromosome. A single transchromosome comprising two or three different immunoglobulin loci provides for genetic linkage of these loci which increases the fraction of transgenic offspring that are useful for making human antibodies. Preferred forms of transchromosomes are those described in detail in Tomizuka, K. et al., 2000, Proc. Natl. Acad. Sci. U.S.A. 97:722-727, Tomizuka, K. et al., 1997, Nature Genetics 16:133-143, and WO 97/07671, WO 98/37757 and WO 00/10383, each of which is incorporated by reference in its entirety for all purposes.

Transchromosomes can also include integrated selectable markers (e.g. neomycin resistance genes) and other sequences not found in the parent intact chromosome. In the event of recombination between a transchromosome and an endogenous mouse chromosome, sequences from the transchromosome are inserted or added to the endogenous mouse chromosome. Transchromosomes can be modified by deletion, translocation, substitution and the like, as described in WO 98/37757, EP 0972445 and WO 00/10383, which are incorporated herein by reference for all purposes. For example, transchromosomes can be fragmented spontaneously in the course of introduction into mouse embryonic stem (ES) cells, fragmented by telomere-directed truncation and/or translocated by Cre/loxP site-specific recombination or similar methods. Such recombination or translocation events can be promoted by specifically inserting recombination

sites (e.g., loxP sequences and others; see, e.g., Abuin, A. and Bradley, A., 1996, Mol. Cell Biol. 16: 1851-1856; Mitani, K. et al., 1995, Somat. Cell. Mol. Genet. 21:221-231; Li, Z. W. et al., 1996, Proc. Natl. Acad. Sci. U.S.A. 93:6158-6162; Smith, A J. et al., 1995, Nat. Genet. 9:376-385; Trinh, K R. and Morrison, S. L., 2000, J. Immunol. Methods 244:185-193; Sunaga, S. et al., 1997, Mol. Reprod Dev. 46: 109-113; Dymecki, S. M., 1996, Proc. Natl. Acad Sci. U.S.A. 93:6191-6196; Zou, YR. et al., 1994, Curr. Biol. 4: 1099-1103; Rudolph, U. et al., 1993, Transgenic Res. 2:345-355; Rickert, R. C. et al., 1997, Nucleic Acids Res. 25:1317-1318). In the case of introduced loxP sites, expression of a transgene encoding the cre recombinase will promote recombination between the two loxP sites. Transchromosomes can also be a fusion chromosome consisting of different chromosome fragments as a result of the translocation described above. Transchromosomes can be **autonomous**. **Autonomous** transchromosomes are distinct from, are noncontiguous with, and are not inserted into the endogenous mouse chromosomes. These **autonomous** transchromosomes comprise telomere and centromere sequences that enable **autonomous** replication. Alternatively, transchromosome sequences can be translocated to mouse chromosomes after introduction into mouse cell nuclei. The endogenous mouse chromosomes include 19 autosomal chromosome pairs and the X and Y chromosomes.

Claims Text - CLTX (3):

2. The transgenic nonhuman mammal of claim 1, wherein the transchromosome is **autonomous**.